

All work done according to standard protocols unless otherwise indicated

September, 2014

Monday, 1st

- Plates grew, picked and grown overnight in the appropriate two antibiotics

Tuesday, 2nd

- Culture miniprepped, cut with e and s to see if both plasmids were there, they were not, picked other colonies

Wednesday, 3rd

- Miniprepped, not both. Cotransformation done again. Rehydrated K608351 heat sensitive promoter and transformed. Prespun media for exosomes collection

Thursday, 4th

- Picked K608351 plates. K145151 rehydrated from kit plate and transformed. Picked cotransformation plates

Friday, 5th

- K608351 cultures prepped and confirmed. Picked k145151 plates. Cotransformation cultures grew, miniprepped and digested again and only one plasmid again.

Saturday, 6th

- RTH mutagenesis of S32-RBS to add universal adaptor (RFC25 suffix). Picked K145151 colonies and grew overnight

Sunday, 7th

- RTH mutagenesis transformed, miniprep of K145151 and digest. Size is good. Miniprepped K145151

Monday, 8th

- S32-B0034 universal mutagenesis plates picked.

Tuesday, 9th

- S32-B0034-Uni cultures miniprepped and sequenced

Wednesday, 10th

- Exosomes isolates and fluorescence characterized.

Thursday, 11th

- Exosome samples characterized using confocal microscope and electron microscope

Monday, 15th

- RTH for s32-B0034-Uni started again. PCR to add RFC25 prefix and suffix to P1010 ccdB.

Tuesday, 16th

- RTH transformed . K105007 rehydrated and transformed

Wednesday, 17th

- Transformations grew, picked and grown overnight

Thursday, 18th

- Miniprep RTH and send for sequencing.
- Miniprep K105007 and started PCR to add NheI and PstI cut sites
- PCR to add NheI and PstI cut sites onto Lamp2b construct started

Friday, 19th

- K608351 and S32-RBS-Uni digested to begin assembly (K608351 upstream and S32-RBS-Uni downstream)
- Gel of digests ran and bands of interest gel extracted
- Extractions nandropped and ligated

Saturday, 20th

- * it looks like the replication origin on the lysis plasmids and the RNA-OUT plasmids are the same. This means they will both be competing for
- K808000-RNA-IN-K112808 and K808000-K112808 digested to be put into pSB2K3